

REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

3

Public reporting burden for this collection of information is estimated to average 1 hour per response gathering and maintaining the data needed, and completing and reviewing the collection of information, including suggestions for reducing this burden, to Washington Headquarters Collection of information, including suggestions for reducing this burden, to Washington Headquarters Collection of information, including suggestions for reducing this burden, to Washington Headquarters Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget.

data sources, spect of this 15 Jefferson

0179

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE

3.

FINAL 15 Aug 92 To 14 Aug 97

4. TITLE AND SUBTITLE

(FY91 EPSCOR) STRUCTURE-ACTIVITY RELATIONSHIPS OF CHLORINATED ALICYCLIC COMPOUNDS IN CATFISH

5. FUNDING NUMBERS

F49620-92-J-0468

3484/E4

61103D

6. AUTHOR(S)

Dr Janice E. Chambers

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mississippi State University
Center for Environmental Health Sciences
College of Veterinary Medicine
Box 9825
Mississippi State MS 39762-9825

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR/NL
110 Duncan Room b115
Bolling AFB DC 20332-8050
Dr Walter Kozumbo

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The scientific goal of this project has been to extend the quantitative structure-activity relationship study of the parent grant, 91-0338, to catfish brain membranes. The binding of [35S]t-butylbicyclophosphorothionate (TBPS) to the Y-aminobutyric acid (GABA) receptor was studied to characterize the binding affinity of the receptor, and to determine the competition of a series of chlorinated alicyclic compounds including chlorinated cyclodiene insecticides and related compounds. These ligand binding studies have indicated the presence of two populations of receptors, with calculated Kd's of 26.10 and 1128.98 nM and Bmax's of 2.95 and 5.14 pmol/mg protein, respectively. All chlorinated alicyclic compounds tested have inhibited TBPS binding except mirex. The IC50's cover a range of 19.97 to 21,177 nM. The IC50's generally correlate with the acute toxicity level for those insecticides reported in the literature.

14. SUBJECT TERMS

19980218 046

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT (U)

18. SECURITY CLASSIFICATION OF THIS PAGE (U)

19. SECURITY CLASSIFICATION OF ABSTRACT (U)

20. LIMITATION OF ABSTRACT (UL)

REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

3

Public reporting burden for this collection of information is estimated to average 1 hour per response gathering and maintaining the data needed, and completing and reviewing the collection of information. Collection of information, including suggestions for reducing this burden, to Washington Headquarters, Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget.

data sources.
aspect of this
15 Jefferson

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE		3. FINAL 15 Aug 92 To 14 Aug 97	
4. TITLE AND SUBTITLE (FY91 EPSCOR) STRUCTURE-ACTIVITY RELATIONSHIPS OF CHLORINATED ALICYCLIC COMPOUNDS IN CATFISH				5. FUNDING NUMBERS F49620-92-J-0468 3484/E4 61103D	
6. AUTHOR(S) Dr Janice E. Chambers					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Mississippi State University Center for Environmental Health Sciences College of Veterinary Medicine Box 9825 Mississippi State MS 39762-9825				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) AFOSR/NL 110 Duncan Room b115 Bolling AFB DC 20332-8050 Dr Walter Kozumbo				10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) The scientific goal of this project has been to extend the quantitative structure-activity relationship study of the parent grant, 91-0338, to catfish brain membranes. The binding of [35S]t-butylbicyclophosphorothionate (TBPS) to the Y-aminobutyric acid (GABA) receptor was studied to characterize the binding affinity of the receptor, and to determine the competition of a series of chlorinated alicyclic compounds including chlorinated cyclodiene insecticides and related compounds. These ligand binding studies have indicated the presence of two populations of receptors, with calculated Kd's of 26.10 and 1128.98 nM and Bmax's of 2.95 and 5.14 pmol/mg protein, respectively. All chlorinated alicyclic compounds tested have inhibited TBPS binding except mirex. The IC50's cover a range of 19.97 to 21,177 nM. The IC50's generally correlate with the acute toxicity level for those insecticides reported in the literature.					
14. SUBJECT TERMS				15. NUMBER OF PAGES	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT (U)		18. SECURITY CLASSIFICATION OF THIS PAGE (U)		19. SECURITY CLASSIFICATION OF ABSTRACT (U)	
				20. LIMITATION OF ABSTRACT (UL)	

Personally Identifiable
Information Redacted

19980218 046

Final Technical Report

The graduate student supported initially under this DEPSCoR agreement was Russell Lloyd Carr, social security number [REDACTED], a United States citizen. He completed his Ph.D. requirements in 1994 in the interdisciplinary Animal Physiology program at Mississippi State University. Prior to enrollment at Mississippi State University, he had received a B.S. in biology and chemistry from Delta State University, Cleveland, MS, in 1987. He received an M.S. in Biological Sciences from Mississippi State University in 1990. He made excellent grades in his doctoral program, and did an excellent job with his research. Since his graduation he has remained in a research capacity at Mississippi State University, as a post-doctoral associate from 1994-1995, and as a Research Toxicologist from 1995 through the present. He currently does research in neurotoxicology, and the training in receptor toxicology he received during his tenure on the AFOSR project was extremely important to the expertise needed for his current research activities.

Following Dr. Carr's graduation, Ms. Terrilyn Theon Atterberry, social security number [REDACTED] a United States citizen and a black female, was placed on this project. She had received her B.S. in Biological Sciences from Mississippi State University, and her M.S. at Mississippi State University in the Animal Physiology program. She is currently enrolled in a Ph.D. program in the Veterinary Medical Sciences program, but may move into the newly established Environmental Toxicology program, since the latter program is more consistent with her career interests. Ms. Atterberry has made exceptionally fine progress with her doctoral program, in both her course work and her research. She will be taking preliminary examinations within the next few months, and will be graduating in 1999. The experience she gained in neurochemistry on the AFOSR project has been a great deal of help to her in her graduate training.

The scientific goal of this project has been to extend the quantitative structure-activity relationship study of the parent grant, 91-0338, to catfish brain membranes. The binding of [35 S]-butylbicyclophosphorothionate (TBPS) to the γ -aminobutyric acid (GABA) receptor was studied to characterize the binding affinity of the receptor, and to determine the competition of a series of chlorinated alicyclic compounds including chlorinated cyclodiene insecticides and related compounds. These ligand binding studies have indicated the presence of two populations of receptors, with calculated K_d 's of 26.10 and 1128.98 nM and B_{max} 's of 2.95 and 5.14 pmol/mg protein, respectively. All chlorinated alicyclic compounds tested have inhibited TBPS binding except mirex. The IC_{50} 's cover a range of 19.97 to 21,177 nM. The IC_{50} 's generally correlate with the acute toxicity level for those insecticides reported in the literature.

Submitted by:

Janice E. Chambers, Ph.D., Principal Investigator
Center for Environmental Health Sciences
College of Veterinary Medicine
Box 9825
Mississippi State University
Mississippi State, MS 39762-9825
601-325-1255; fax 601-325-1031
chambers@cvm.msstate.edu

TABLE 1

CHARACTERISTICS OF [³⁵S]TBPS SPECIFIC BINDING TO
BRAIN P₂ MEMBRANES OF CHANNEL CATFISH

	K_d^1 (nM)			B_{max} (pmol/mg P)		
High Affinity Site	26.40	±	5.70	2.95	±	0.57
Low Affinity Site	1128.98	±	263.92	5.14	±	0.83

¹Values Expressed as Mean ± S.E.

TABLE 2

INHIBITION OF TBPS BINDING TO CATFISH BRAIN
GABA RECEPTORS BY VARIOUS ORGANOCHLORINE
COMPOUNDS

Compound	IC ₅₀ ¹ (nM)		
12-Ketoendrin	19.97	±	3.93
Photoheptachlor Epoxide	20.82	±	4.65
Photoheptachlor	25.23	±	6.21
Telodrin	39.80	±	6.25
Endrin	89.83	±	14.74
Photooxychlordane	122.48	±	18.61
Photo α -Chlordane	186.03	±	30.71
Oxychlordane	218.69	±	57.20
Isodrin	310.56	±	60.16
Heptachlor Epoxide	330.12	±	33.93
Dieldrin	592.49	±	104.95
Lindane	888.81	±	23.25
Heptachlor	2073.74	±	148.05
Aldrin	2140.28	±	383.43
Chlordene	10201.91	±	1270.25
Chlordecone (Kepone)	21177.90	±	2110.87
Mirex	No Inhibition		

¹IC₅₀ Values Expressed as Mean ± S.E.

TABLE 3

IC₅₀'S OF [³⁵S]TBPS BINDING INHIBITION TO
CATFISH BRAIN GABA RECEPTORS AND LC₅₀'S OF
VARIOUS CHLORINATED ALICYCLIC COMPOUNDS IN
CHANNEL CATFISH¹

Compound	IC ₅₀ (nM)	96 hr LC ₅₀ (μG/L)
Endrin	89.83	0.29
Dieldrin	592.49	4.50
Heptachlor	2073.74	25.00
Aldrin	2140.28	53.00
Chlordecone	21177.90	225.00

¹ LC₅₀ data from Mayer and Ellersieck (1986).

Figure 1
Saturation Plot for TBPS Binding

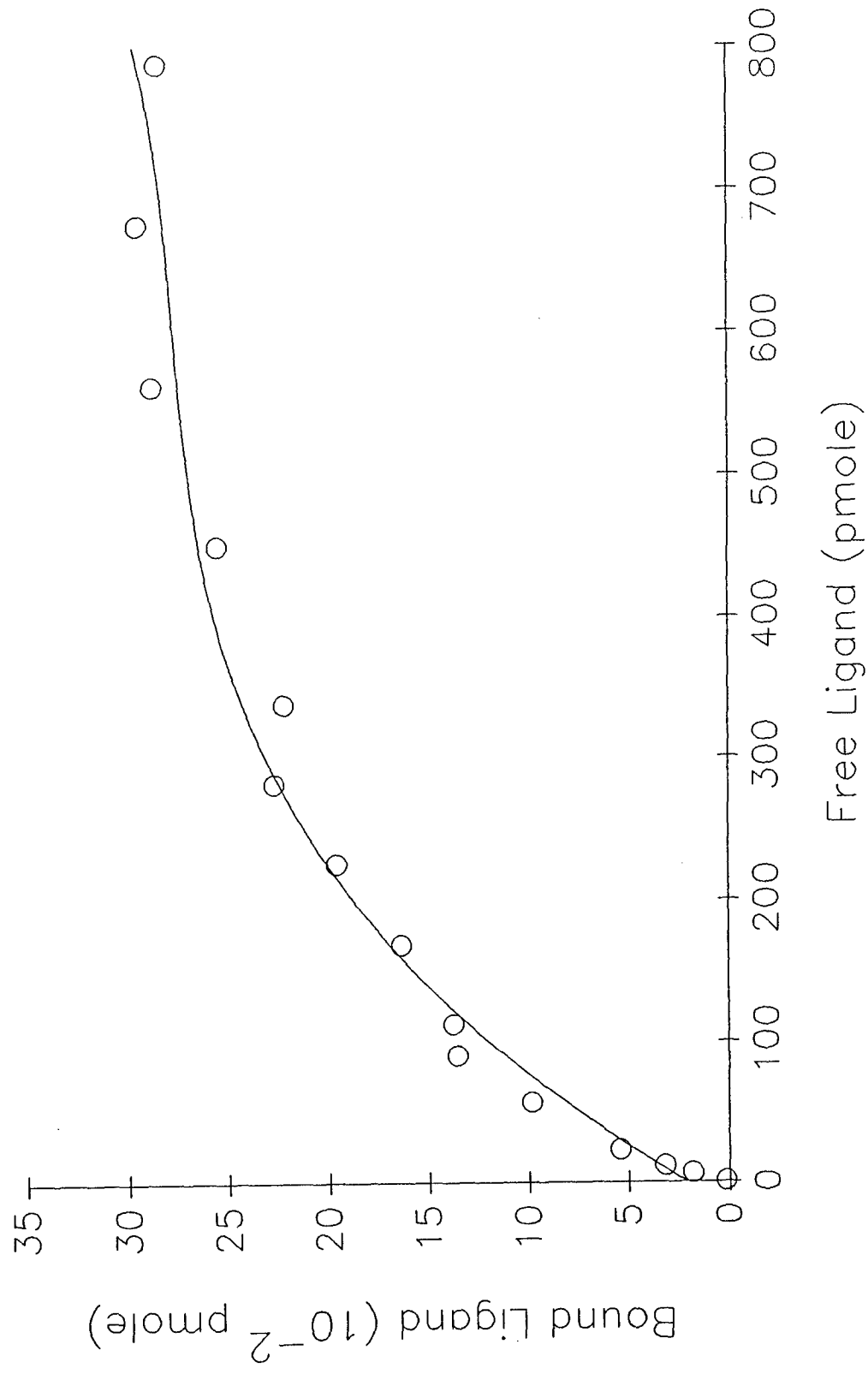


Figure 2
Scatchard Plot for TBPS Binding

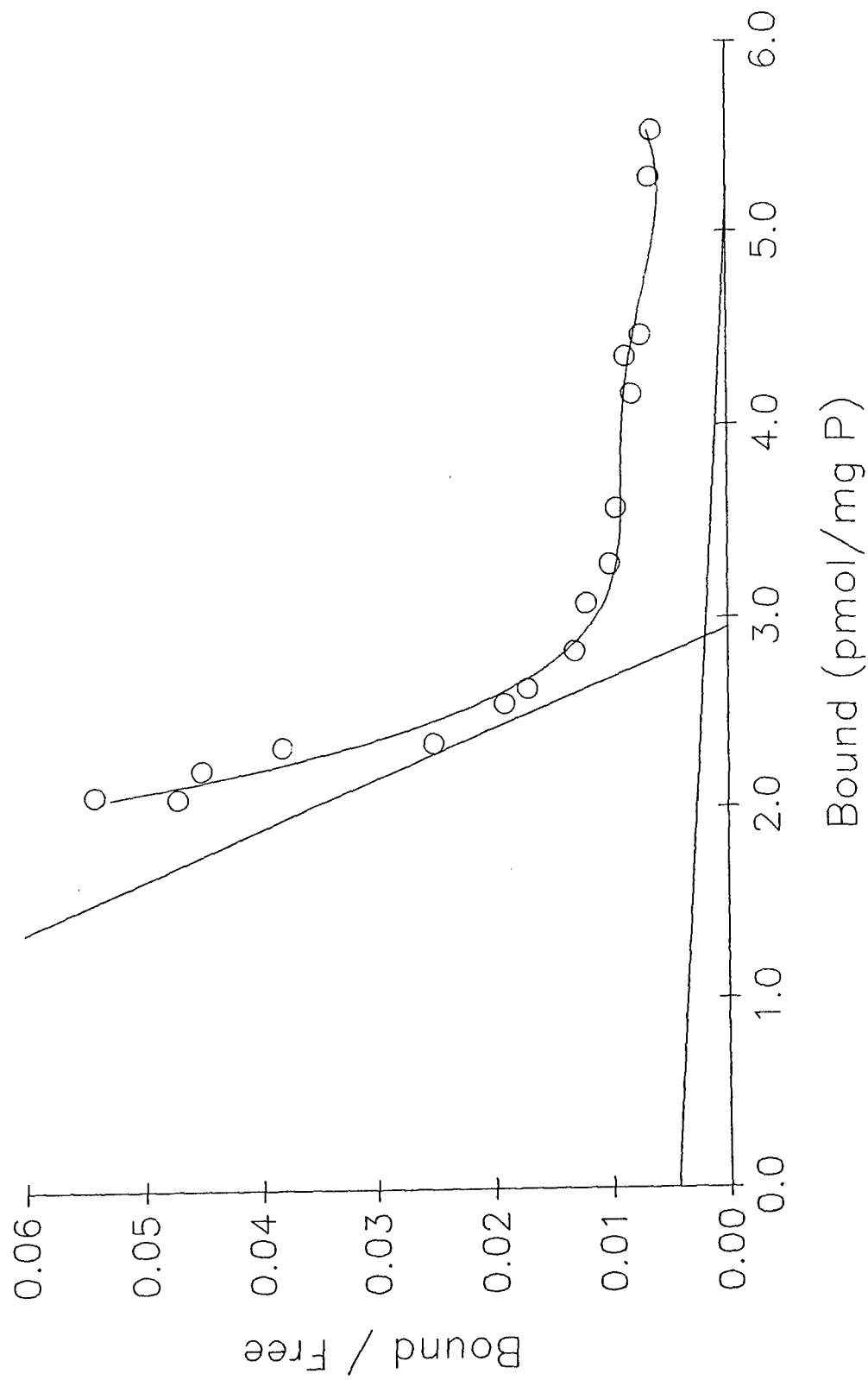


Figure 3
Inhibition Curves

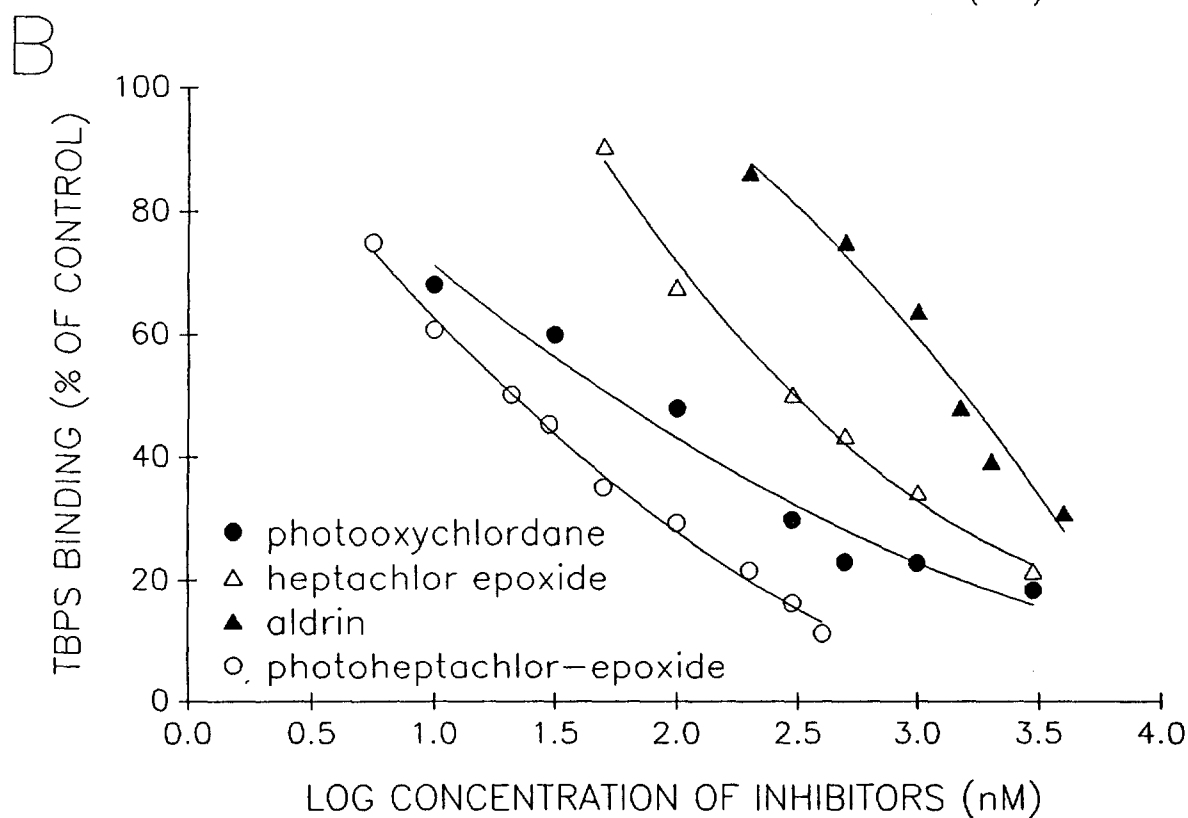
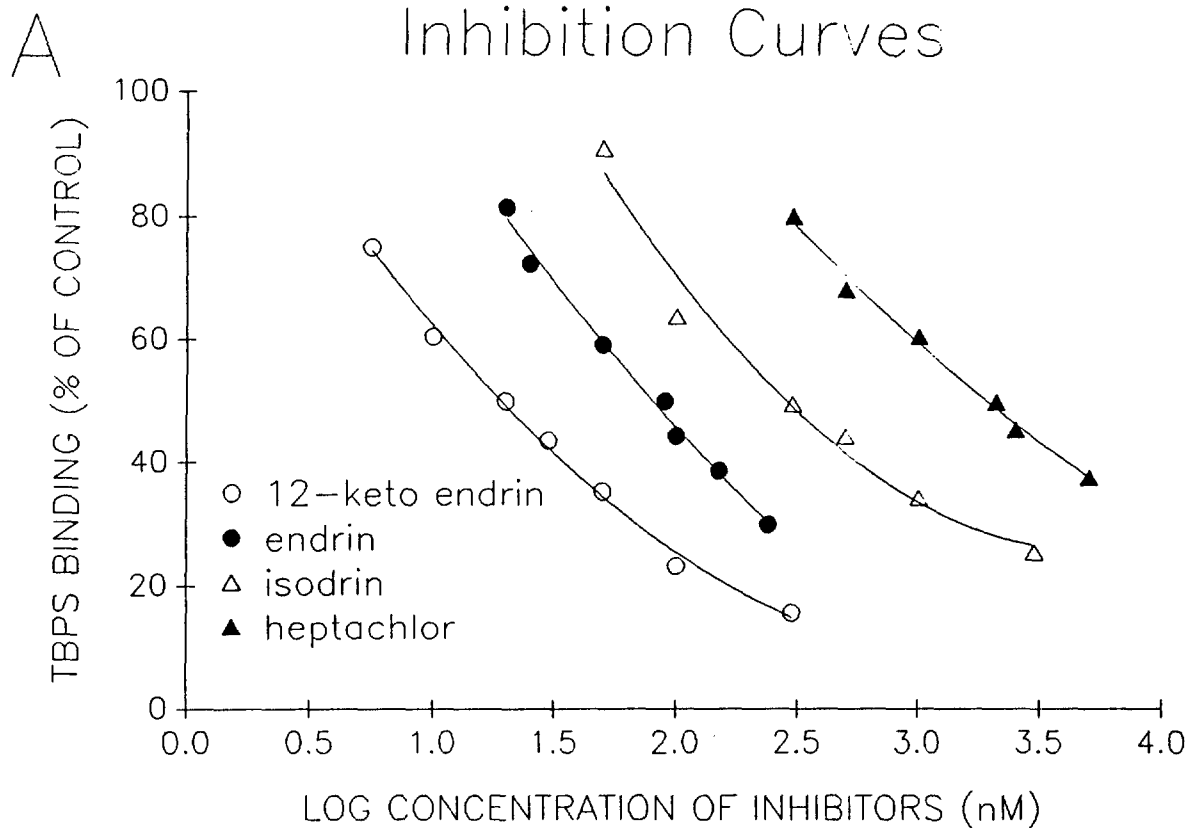


Figure 4

Inhibition Curves

